A STUDY ON AEROBIC BACTERIA IN PATIENTS WITH

20-40% BURNS IN A TERTIARY CARE CENTRE

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CERTIFICATE

Certified that the dissertation entitled "A STUDY ON AEROBIC BACTERIA IN PATIENTS WITH 20 – 40% BURNS IN A TERTIARY CARE CENTRE is a bonafide work done by Dr. T.SABEETHA, Postgraduate, Institute of Microbiology, Madras Medical College Chennai, under my guidance and supervision in partial fulfillment of the regulation of the Tamil Nadu Dr. M.G.R Medical University for the award of M.D. Degree, Branch-4 (Microbiology) during the academic period of May 2004 to March 2007.

Dr. A. LALITHA, M.D.,D.C.P.,
Director & Professor
Institute of Microbiology,
Madras Medical College,
Chennai-600003

DEAN

Dr. KALAVATHY PONNIRAIVAN, B.Sc., M.D.,
Madras Medical College and Government General Hospital,
Chennai-600003.

DECLARATION

I declare that the dissertation entitled **"A STUDY ON AEROBIC BACTERIA IN PATIENTS WITH 20-40% BURNS IN A TERTIARY CARE CENTRE"** submitted by me for the Degree of M.D., is the record work carried out by me during the period of May 2004 to March 2007 under the guidance of **Dr. G.SASIREKHA,** M.D., Professor of Microbiology, Institute of Microbiology, Madras Medical College, Chennai and has not formed the basis of any Degree, Diploma, Associateship, Fellowship titles in this or any other University or other similar institution of higher learning.

Signature of the candidate

Place :Chennai
Date :

(Dr.T.SABEETHA)

Signature of the Guide
Dr. G.SASIREKHA, M.D.,
Professor
Institute of Microbiology,
Madras Medical College
Chennai

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CONTENTS

INTRODUCTION

Patients with burn injuries are highly susceptible for infection as a result of disruption of the normal skin barrier and accompanying depression of immune response.

The burn surface contains a large amount of necrotic tissue and the protein rich wound exudates provides a rich growth medium. So, following the initial period of shock, infection is the major complication and about 75% of the mortality associated with burn injuries is related to infection. In addition, overcrowding in Burns Unit is an important cause of cross-infection which necessitates a periodic monitoring of bacterial species and their antibiogram. The organisms are mainly derived from the patients gastro intestinal and upper respiratory tracts as well as from the hospital environment. [1, 2]

It is crucial for every burn institution to determine the specific pattern of burn wound microbial colonization, the time-related changes in the dominant flora, and the antimicrobial sensitivity profiles. This would enable early treatment of imminent septic episodes with proper empirical systemic antibiotics, without waiting for culture results, thus improving the overall infection-related morbidity and mortality.

Individual organisms are brought into the burns ward in the wounds of new patient. These organisms persists as dominant flora in the burn treatment unit until new organisms are introduced. So

periodical study of the microbial profile and antibiogram is necessary.

Infection, the risk of which is proportional to the extent of injury, continues to be the predominant determinant of outcome in thermally injured patients despite improvements in overall care in general and wound care in particular. The control of invasive burn wound infection through the use of effective topical chemotherapy, prompt surgical excision, and timely closure of the burn wound has resulted in unsurpassed survival rates. Even so, infection remains the most common cause of death in these severely injured patients.

Alterations in the protocols of burn wound management and the introduction of new topical and systemic antimicrobial agents eventually influence the nature of the microbial flora of the ward [3]

Most of the infections are thought to be of nosocomial origin wherein hand and clothing of attending staff has been implicated in many cases. In specialized units of the hospital with high infection rates, the infective agents vary with time and is unique to different hospitals.

In India where burns usually occur in the lower socioeconomic strata and in a productive age group-rational antibiotic therapy according to the prevalent strains of organisms should help in reducing the mortality and morbidity associated with burns. The analysis of the isolates and their sensitivity patterns helps to formulate an institutional drug policy for the patients admitted in Burn Unit.

REVIEW OF LITERATURE

Improvements in burn care originated in specialized units specifically dedicated to the care of burned patients. The interactive multidisciplinary team has proven to be the least expensive and most efficient method of treating major burn injury, of which the initial acute care is only a small part of the total treatment. Burn patients often require years of supervised rehabilitation, reconstruction, and psychosocial support. Omission of any step in the treatment regimen by any of the burn team members, including the burn surgeon, nurses, therapists, nutritionists, or psychosocial support staff, can result in less than optimal outcomes.

EPIDEMIOLOGY

A study conducted in United States says that approximately 1.1 million individuals annually are burned seriously enough to seen health care; about 45,000 die. More than 90% of burns are preventable; nearly one half are smoking related or due to substance abuse[4,5].

The burn size associated with a 50% mortality rate has increased from 30% of the total body surface area (TBSA) to greater than 80% TBSA in otherwise healthy young adults[6,7]. The quality of burn care is no longer measured only by survival, but also by long-term function and appearance. Although small burns are not usually life threatening, they need the same attention as larger burns to achieve optimal outcomes.

As with other forms of trauma, burns frequently affect children and young adults. In children under 8 years of age, the most common burns are scalds, usually from the spilling of hot liquids[8]. In older children and adults, the most common burns are flame-related, usually the result of house fires, the ill-advised use of flammable liquids as accelerants, or smoking-or alcohol-related[9]. Chemicals or hot liquids, followed by electricity, and then molten or hot metals most often cause work-related burns[10].

ETIOLOGY OF BURN INJURY

SCALD BURNS

Scalds, usually from hot water, are the most common cause of burns in civilian practice. Water at 140°F (60°C) creates a deep partial-thickness or full-thickness burn in 3 seconds. At 156°F (69°C), the same burn occurs in 1 second[11]. Exposed areas of skin tend to be burned less deeply than clothed areas, as the clothing retains the heat and keeps the hot liquid in contact with the skin for a longer period of time. Immersion scalds are always deep, severe burns[12].

FLAME BURNS

Flame burns are the second most common mechanism of thermal injury. Smoking-related fires, improper use of flammable liquids, motor vehicle collisions, and ignition of clothing by stoves or space heaters also are responsible for flame burns.

FLASH BURNS

Flash burns are next in frequency. Explosions of natural gas, propane, butane, petroleum distillates, alcohols, and other combustible liquids, as well as electrical areas cause intense heat for a brief time period[13,14]. At least some areas of flash burns often heal without requiring extensive skin grafting, but the burns generally cover a large TBSA, and in an explosive environment may be associated with significant thermal damage to the upper airway.

CONTACT BURNS

Contact burns result from contact with hot metals, plastic, glass, or hot coals. They are usually limited in extent, but are invariably deep. It is common for patients involved in industrial accidents to have associated crush injuries because these accidents are commonly caused by contact with presses or other hot, heavy objects[15]. Motor vehicle and motorcycle collisions may leave victims in contact with hot engine parts[16]. The exhaust pipes of motorcycles cause a characteristic burn of the medial lower leg, that although small, usually requires excision and grafting.

Causes

Flame	Damage from superheated, oxidized air
Scald	Damage from contact with hot liquids
Contacts	Damage from contact with hot or cold solid materials
Chemicals	Contact with noxious chemicals
Electricity	Conduction of electrical current through tissues

BURN DEPTH

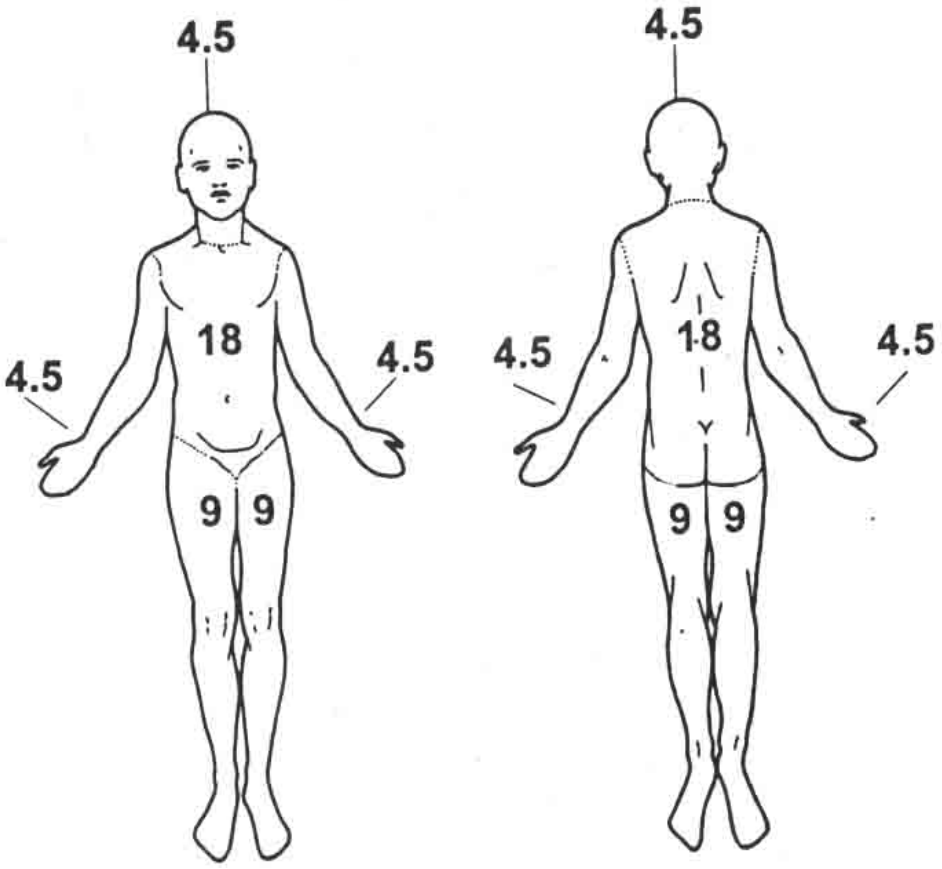
The depth of burn varies depending on the degree of tissue damage. Burn depth is classified into degree of injury in the epidermis, dermis, subcutaneous fat, and underlying structures.

Depths

First degree	Injury localized to the epidermis
Superficial second degree	Injury to the epidermis and superficial dermis
Deep second degree	Injury through the epidermis and deep into the dermis
Third degree	Full-thickness injury through the epidermis and dermis into the subcutaneous fat.
Fourth degree	Injury through the skin and subcutaneous fat into underlying muscle or bone

BURN SIZE

Determination of burn size estimates the extent of injury. Burn size is generally assessed by the “rule of nines”. In adults, each upper extremity and the head and neck are 9% of the TBSA., the lower extremities and the anterior and posterior trunk are 18% each, and the perineum and genitalia are assumed to be 1% of the TBSA. Children have a relatively larger portion of the body surface area in the head and neck, which is compensated for by a relatively smaller surface area in the lower extremities. Infants have 21% of the TBSA in the head and neck and 13% in each leg, which incrementally approaches the adult proportions with increasing age.



Body surface area diagram. This figure depicts the relative percentage of the total body surface area of defined anatomic areas

It would seem likely that the larger the burn wound, the more likely it would be contaminated and colonized with microorganisms. In a prospective study of 53 pediatric patients with burns in which the data were analyzed by multivariate analysis, Fleming and coworkers showed a significant relationship between colonization of burn wounds with microorganisms from patients fecal flora and the size of their burn wounds[17]. Again, it is likely that risk factors for colonization also place the burn wound at greater risk for infection.

Berkow Digram to Estimate Burn Size Based on Area of Burn in an Isolated Body Part in Percentage.

Area	0-1yr	1-4yr	5-9yr	10-14yr	15-18yr	adult
Head	19	17	13	11	9	7
Neck	2	2	2	2	2	2
Anterior trunk	13	13	13	13	13	13
Posterior trunk	13	13	13	13	13	13
Right buttock	2.5	2.5	2.5	2.5	2.5	2.5
Left buttock	2.5	2.5	2.5	2.5	2.5	2.5
Genitalia	1	1	1	1	1	1
Rt upper arm	4	4	4	4	4	4
Lt upper arm	4	4	4	4	4	4
Rt lower arm	3	3	3	3	3	3
Lt lower arm	3	3	3	3	3	3
Rt hand	2.5	2.5	2.5	2.5	2.5	2.5
Lt hand	2.5	2.5	2.5	2.5	2.5	2.5
Rt thigh	5.5	6.5	8	8.5	9	9.5
Lt thigh	5.5	6.5	8	8.5	9	9.5
Rt leg	5	5	5.5	6	6.5	7
Lt leg	5	5	5.5	6	6.5	7
Rt foot	3.5	3.5	3.5	3.5	3.5	3.5
Lt foot	3.5	3.5	3.5	3.5	3.5	3.5

In a retrospective study in which data were analyzed by multiple regression analysis, Merrell and colleagues found a significant relationship between burn wound size and subsequent occurrence of fatal sepsis[18]. Fifty-four percent of the fatalities were due to burn shock. Graves and associates also observed a significant relationship between burn wound size and infection in a retrospective study[19].

BURN PREVENTION

More than 90% of all burns are preventable, and ongoing prevention and education efforts seem to be the most effective means to impact burn incidence. Using flame-resistant sleepwear for children, smoke detectors, required in all residential rental units and new construction, have likely contributed to decreased burn severity and mortality[20].

BURN CENTER REFERRAL CRITERIA

1. Partial-thickness and full-thickness burns totaling greater than 10% TBSA in patients under 10 or over 50 years of age.

2. Partial-thickness and full-thickness burns totaling greater than 20% TBSA in other age groups.

3. Partial-thickness and full-thickness burns involving the face, hands, feet, genitalia, perineum, or major joints.

4. Full-thickness burns greater than 5% TBSA in any age group.

5. Electrical burns, including lightning injury.

6. Chemical burns.

7. Inhalation injury.

8. Burn injury in patients with preexisting medical disorders that could complicate management, prolong the recovery period, or affect mortality.

9. Any burn with concomitant trauma (e.g., fractures) in which the burn injury poses the greatest risk of morbidity or mortality. If the trauma poses the greater immediate risk, the patient may be treated initially in a trauma center until stable, before being transferred to a burn center. The physician's decision should be made with the regional medical control plan and triage protocols in mind.

10. Burn injury in children admitted to a hospital without qualified personnel or equipment for pediatric care.

11. Burn injury in patients requiring special social, emotional, and/or long-term rehabilitative support, including cases involving suspected child abuse.

ETIOLOGY OF BURN WOUND INFECTION

Both the nature of the burn wound and microorganism-specific factors influence the rate of microbial proliferation in and penetration of

the burn eschar. Burn tissue, rich in coagulated protein and well hydrated by the trans-eschar movement of fluid and serum creates an excellent microbial culture medium. The eschar is avascular owing to thermal thrombosis of nutrient vessels, limiting both the delivery of systemically administered antibiotics and the migration of phagocytic cells into the burned tissue. Bacterial proliferation in the wound may also be enhanced by such factors as wound maceration, pressure necrosis, and wound desiccation with neo-eschar formation. In addition, secondary impairment of blood flow to the wound may further predispose the patient to invasive infection by curtailing the delivery of oxygen, nutrients, and phagocytic cells to the viable subeschar tissue.

Without the application of topical antimicrobial agents, the density of bacteria grows progressively, and the microorganisms penetrate the eschar by migration along sweat glands and hair follicles until the eschar nonviable tissue interface is reached. Further microbial proliferation occurs in the subeschar space, enhancing the lysis of denatured collagen and sloughing of the eschar. If the density and invasiveness of the microorganisms exceed the defense capacity of the host, proliferating organisms in the subeschar space may invade the underlining viable tissue, leading to invasive burn wound infection and even systemic spread to remote tissues and organs.

Certain strain-specific factors also appear to be important in the pathogenesis of invasive burn wound infection. The production of enzymes, such collagenase, elastase, protease, and lipase, may enhance the organisms ability to penetrate the eschar. Moreover,

bacterial motility and antibiotic resistance appears to be important in the development of invasive infection. Effective topical antimicrobial chemotherapy limits intraeschar bacterial proliferation and the attendant risk of invasive infection.

HOST IMMUNE FUNCTION

Thermal injury initiates a deleterious pathophysiologic response in every organ system, with the extent and duration of organ dysfunction proportionate to the size of the burn. Direct cellular damage is manifested by coagulation necrosis, with the depth of tissue destruction determined by the duration of contact and the temperature to which the tissue is exposed. Following burn, the normal skin barrier to microbial penetration is lost, and the moist, protein-rich avascular eschar of the burn wound provides an excellent culture medium for microorganisms. While destruction of the mechanical barrier of the skin contributes to the increased susceptibility to infection, postburn alterations in immune function may also be of significant importance. Every component of the humoral and cellular limbs of the immune system appears to be affected after thermal injury; the magnitude and duration of dysfunction are proportional to the extent of injury.

Alterations in lymphocyte subpopulations, including reversal of the normal ratio of T-helper cells to T-suppressor cells, have been described[21,22]. Delayed hypersensitivity reactions and peripheral blood lymphocyte proliferation in the mixed lymphocyte reaction are both inhibited following burns. Alterations in interleukin-2 (IL-2) production

and IL-2 receptor expression by lymphocytes have been observed with burn injury; a direct correlation has been established between the extent of the burn and the decline of IL-2 production by peripheral lymphocytes[23].

Increased numbers of circulating B lymphocytes are evident early in the postburn course; however, serum immunoglobulin G (IgG) levels decline after burn injury and gradually return to normal over the succeeding 2 to 4 weeks. The association of higher numbers of circulating B cells but reduced serum levels of IgG suggests a defect in the ability of B cells to generate a normal response after burn injury[24]. Similar findings have been observed in IgM producing cells isolated from murine mesenteric lymph nodes and spleens following burns[25]. Exogenous administration of IgG to burn patients has been shown to promptly restore IgG levels to normal but exerts no demonstrable effect on the incidence or outcome of infections[26].

RISK FACTORS FOR INFECTION

The extent of burn injury (i.e., TBSA) is one of the major determinants of overall outcome, and the incidence of infection correlates with burn severity.[27] Children appear to be more susceptible to systemic infection than adults for any given size burn.[28] However, burns involving less than 20% TBSA in otherwise healthy individuals are rarely associated with life-threatening infection. The presence of an inhalation injury strongly correlates with infection, particularly pneumonia. Premorbid diabetes significantly increases infection rates

following thermal injury, especially when coupled with poor glycemic control[29] .

In addition to the risk of infection associated with the wound itself, catheters and other invasive devices increase risk[30]. The use of silver-impregnated devices, including central lines and Foley catheters, may reduce catheter-related infection risk. Other causes of morbidity and mortality in patients with burn injuries are inhalation injuries and pneumonia.

CLINICAL MANIFESTATIONS AND DIAGNOSIS

CLINICAL SIGNS OF BURN WOUND INFECTION

1 Focal dark brown or black discoloration of wound

2 Conversion of second degree burn to full-thickness necrosis

3 Degeneration of wound with neo-eschar formation

4 Unexpectedly rapid eschar separation

5 Hemorrhagic discoloration of subeschar fat

6 Violaceous or erythematous edematous wound margin

7 Metastatic septic lesions in unburned skin or distant organs

Many of the physiologic criteria defining sepsis are noninfectious sequelae of postinjury hypermetabolism. Hyperthermia, tachyeardia, increased ventilation, and high cardiac output, indicative of sepsis as well as a hyperdynamic-hypermetabolic state, are part of the normal response to major burns in otherwise healthy patients.

Body temperature regulation is altered in burn patients, and is partially dependent upon environmental conditions. Hyperthermia (>38.5°C) is routinely present following thermal injury, particularly in children, and is a poor indicator of infection.[31,32] Conversely, hypothermia commonly heralds sepsis, usually due to gram-negative organisms. Leukocytosis in the burn patient is also nonspecific. As long as large wounds remain open, variable elevations in leukocyte counts are common. Thrombocytosis is invariable following major thermal injury, whereas thrombocytopenia is a fairly reliable manitestation of sepsis.

WOUND INFECTION

All burn wounds become colonized by 72 hours after injury with the patient's own flora or with endemic organisms from the treatment facility. Bacteria colonize the surface of the wound and may penetrate the avascular eschar.

By definition, burn wound invasion, is a histologic diagnosis where microscopic evidence of invasion of non-burned tissue with bacteria is present and bacteremia imminent. Unfortunately, burn wound biopsies are expensive, invasive because a section of unburned tissue needs to be included with the biopsy, and associated with considerable variability between adjacent sites of the burn wound[33]. These facts together with more aggressive wound debridement, newer topical antimicrobials, and improved nutritional support and intensive care have limited the use of burn wound biopsy in many burn centers[34].

Swab cultures are helpful to identify potential pathogens colonizing the wound, and they provide valuable information to guide prophylactic perioperative systemic antibiotic selection for wound debridement. Whereas for anaerobic cultures exudates, swabs from burns, wounds and skin abscesses are generally unaceeptable for anaerobic cultures[52].

Types of bacteria that colonize and infect burn patients and their antibiotic sensitivity are highly variable between burn units and highly influenced by topical antimicrobials and wound care policies as well as systemic antibiotic use. Revathi et al. described their experience in 600 burn patients with infections[35] which was similar to many burn units where the most common and severe infections were caused by *Pseudomonas spp*., than *Staphylococcus aureus* followed by other gram negative organisms that include *Klebsiella spp*., *Acinetobacter spp*., *Escherichia coli*, *Enterococcus faecalis*, *Proteus spp*., and others. A recent comparison between two, 10-year periods of gram negative isolates in pediatric burn patients showed that in the 1990s, *Ps*. aeruginosa accounted for 35.2% of gram negative organisms from infection sites compared to 33.6% in the 1980s. Recently, *Acinetobacter spp*., replaced *Klebsiella spp*., as the second most common bacteria causing infections in pediatric burn patients[36]. In a recent overview of wound isolates in United Kingdom burn centers, an increasingly prevalent *Acinetobacter* has been reported[37].

Serious infections caused by *Pseudomonas aeruginosa* remain a common complication in thermally injured patients contributing

substantially to burn morbidity and mortality. In a survey of 176 burn care centers in North America; *Pseudomonas* species was considered the most serious cause of the life threatening infections in the thermally injured patients[38]. Similarly, in 1985 a 25 year review of *Pseudomonas* bacteremia in burn patients by McManus et al. documented an overall burn mortality of 77% with *Ps. aeruginosa* bacteremia that was 28% above predicted[39]. Despite advancements in medical and surgical care of burn patients, no significant improvement in mortality was documented over the 25-year period in which burn patients cared for in their institution[39].

Ps. aeruginosa is an opportunistic gram-negative pathogen which produces many exoproducts including elastase, alkaline protease, hemolysin, exotoxin A, exoenzyme S and together with its heterogeneous lipopolysaccharide mediate much of its virulence[40]. *Pseudomonas* organisms possess flagellum rendering it motile, a morphologic feature considered important in virulence in burn patients[41]. However, adherence of the bacterium to both biologic cell membranes and inert surfaces is mediated through pili or fimbriae and by the production of large amounts of exopolysaccharides which are strongly anionic linear chains of β-D-mannuronic and α-1-guluronic acids that bind water and form gels[42]. This alginate or slime production mediates adhesion in the mucoid non-motile form of *Pseudomonas* , where together with specific ion binding proteins such as the iron binding siderophores, pyochelin and pyoveridin, it can extract iron and other nutrients from its environment and thrive in microcolonies[40]. The

production of alginate and many cell surface enzymes including penicillinases makes the organism very difficult to treat or eradicate and predisposes the burn wounds and medical equipment and devices in burn units to *Pseudomonas* infection and contamination.

The gram negative bacteria including *Klebsiella, E. coli* and *Acinetobacter* as well as *Pseudomonas* are the major causes of mortality in burn center. McManus et al. in 1985 suggested that 10% of all burn patients develop *Pseudomonas* bacteremia which carries a mortality rate of 80%[39,43] and the risk of *Pseudomonas* infection increases substantially in burns>30%. Both for cystic fibrosis and burns, *Pseudomonas* morbidity and mortality can be significantly reduced by measures taken to avoid nosocomial infection or to delay the onset of infection as long as possible[44]. Avoidance of nosocomial *Pseudomonas* infections is likely of major significance not for the small superficial of clean wound in younger patients where the risk of mortality is low, but in larger or deeper wounds or in hosts with increasing age, inhalation of other risk factors which put them at risk of death from burn injury particularly if they become infected early in their course with an antibiotic resistant gram negative organisms such as *Pseudomonas* [45].

A prospective study was carried out on 70 burned patients admitted to the Burn Unit, Ain Shams University Hospital, Cairo, with the aim to verify the pattern of microbial colonization of burn wounds. Throughout the study period starting from 1 June 1999 till 31 May 2001, 281 sampling procedures (surface swabs) were performed from the burn wounds. A total of 301 microbial isolates were grown in cultures,

Eight different species of bacteria, and only one species of Candida (*C. albicans*) were detected. There was no incidence of recovery of anaecrobic microorganisms. Their results revealed that the most frequent isolate was *Ps. aeruginosa* (21.6%), followed by *Klebsiella pneumoniae* (15.2%), then *Escherichia coli* (13.6%), *Staphylococcus aureus* (13.2%), coagulase-negative *Staphylococci* (11.6%) *Streptococcus pyogenes* (8.3%) *Enterobacter species* (6.6%), and lastly *Streptococcus faecalis* and *Candida albicans* (5.9 and 3.6%, respectively). Studying the time-related changes in burn wound microbial colonization showed an initial predominance of gram-positive cocci upon admission (70.7%) over gram-negative bacilli 27.6%[46]. During the first 5 days gram-negative bacilli started to predominate (55.7%) over gram positive cocci (40.3%). Burn wound sampling performed starting from the sixth day onwards revealed further prevalence of gram negative bacilli (72.7%) over gram positive cocci (22.7%)

In specialized units of the hospital with high infection rates, the spectrum of infective agents varies with time and is unique to different hospitals. *Acinetobacter spp*. are rapidly emerging as an important pathogens predominantly in many units such as intensive care units, burn units and surgical wards across the globe including India[47-50]. Emergence of *Acinetobacter spp*. with the added problem of fast emerging multiple resistance is becoming a serious therapeutic problem as increased mortality has been reported[48]. The increase of multi-drug resistant extended spectrum beta-lactamase (ESBL) producing strains among the clinical isolates has further limited the therapeutic options[50]. It is therefore necessary to carry out periodic reviews of patterns of

isolation and susceptibility profiles of microorganisms infecting burn wounds in order to modify the preventive and therapeutic strategies.

A retrospective study of major aerobic bacterial isolates from pus/wound swabs taken from patients admitted to the burn unit at Govt. Medical Collage Hospital, Chandigarh, India, over a period of 5 years (June 1997-May 2002) was undertaken. The study was carried out to determine the bacterial profile and antimicrobial susceptibility of the isolates and to describe the change in trends over the study period. The pus/wound swabs yielded very high culture positivity (96%) for 665 total isolates. *Pseudomonas aeruginosa* was found to be most common isolate (59%) followed by *Staphylococcus aureus* (17.9%). *Acinetobacter spp*. (7.2%), *Klebsiella spp*. (3.9%), *Enterobacter spp*. (3.9%), *Proteus spp*. (3.3%) and others (4.8%). Although Ps. aeruginosa continued to remain the predominant isolate over the five years, a constant and significant increase in the incidence of *Acinetobacter spp*. was found. Amikacin was found to be the most effective drug against gram negative bacteria, however, resistance to it was significantly increased over 5 years. For S. aureus and Ps. aeruginosa netilmicin and piperacillin were found to be the most effective drugs. Most of the isolates showed high level resistance to antimicrobial agents[51].

Nasser et.al (2003) study revealed coagulase-negative Staphylococci were recovered at a frequency of 11.6%. this relatively low incidence is consistent with many previous reports on burn wound colonization in which the pathogenicity of this organism has been

questioned. Vindenes et.al study revealed higher rates of recovery of CONS in the wound of burned patients. In view of the immunocompromise status of critically-ill burned patients, such centers have consistently stressed that CONS should be considered a significant pathogen.

Liedberg et al., emphasized septicaemia as a common cause of death[55].

Tumbusch et al., reported that more alarming upsurge in septicaemia due to gram negative bacteria. This has been predominantly due to *Pseudomonas aeruginosa* but occasionally *Klebsiella & Escherichia* [56].

Rosen F. S. points out that destruction of large number of bacteria with release of endotoxin probably occurs in the burns wound and in other sites of infection. The circulating bacterial endotoxin may be responsible for the severe physiological disturbance of *Pseudomonas* septicaemia in addition to irreversible shock severe leucopenia and profound hypothermia or hyperpyrexia are consistent effects of lethal doses of endotoxins in a variety of species[57].

PREVENTION OF BURN WOUND INFECTION

Progress in the general care of the critically burned patient has included a significant emphasis on the prevention of infectious complications. These efforts have focused mainly on the areas of environmental control (through single-bed rooms and other forms of

isolation) and topical antimicrobial prophylaxis of the burn wound. Effective infection control programs are essential to reducing the exposure of patients in critical care units to nosocomial pathogens. Such control includes strictly enforced hand washing, gowning, and gloving policies. When new endemic microbial strains are identified, increased efforts to prevent patient-to-patient spread and unit environmental contamination may be accomplished using cohort patient care techniques. Cohort care, which entails the assignment of patient care personnel in teams to provide care for only a specific patient or only patients colonized or infected with a targeted organism limits the spread of, and can even eliminate, antibiotic-resistant endemic organisms[58].

Effective infection control programme for burn centers should include scheduled microbial surveillance of colonization of patients, environmental hygiene monitoring procedures, biopsy assessment of the microbial status of the burn wound as necessary, monitoring of the incidence and causes of infection, and timely review of culture and clinical data by an infection control committee.

Early removal of devitalized tissue prevents wound infections and decreased inflammation associated with the wound. In addition, it eliminates small-colonized foci, which are a frequent source of transient bacteremia.

Topical and systemic antimicrobial therapy has significantly diminished the incidence of invasive burn wound sepsis. Perioperative

antibiotics clearly benefit patients with injuries greater than 30% TBSA burns.

BURN WOUND HYGIENE AND TOPICAL ANTIMICROBIAL THERAPY

Care of the burn wound begins at the accident scene by covering the wounds with clean sheets or blankets to preserve body temperature and prevent continued environmental exposure. The burns are gently cleansed with a surgical detergent disinfectant, and nonviable epidermis is debrided. Bullae should be excised and body hair shaved from the area of thermal injury beyond the margin of normal skin. The patient is placed in a clean bed, and bulky dressings may be positioned beneath the burned parts to absorb the often copious serous exudates. These dressings should be changed when they become saturated or soiled and patients should be turned frequently to prevent maceration of burned and unburned skin.

Burn injury treatment includes the use of both systemic and topical therapies, in addition to wound debridement, immune support, and proper nutrition.[59]. Systemic antibiotics are administered to prevent development of infection; however, if sepsis occurs, antibiotics are continued until 72 hours after the infection has been eradicated[60]. Topical antimicrobial agents are used to prevent microbial growth within the burn wound.

The three agents with proven broad-specturm antimicrobial activity when applied to the burn wound are silver sulfadiazine (SSD), mafenide acetate, and silver nitrate. SSD is the most common agent

used in burn centers and has antifungal properties in addition to good bacterial coverage[61]. However, SSD does not penetrate eschar. Only mafenide acetate is able to penetrate eschar, and it is the only agent capable of suppressing dense subeschar bacterial proliferation. The main disadvantage of mafenide acetate is its carbonic anhydrase inhibition, which may interfere with renal buffering mechanisms. Bicarbonate is consumed, chloride is retained, and the resulting hyperchloremic metabolic acidosis is compensated for by an increase in ventilation and subsequent respiratory alkalosis. However, this is typically of little clinical consequence. Silver nitrate must be used before bacteria have penetrated the wound. Its disadvantages are the associated electrolyte imbalances (e.g., hyponatremia).

Topical antimicrobial creams are usually used with closed dressings. This provides for greater patient comfort and less dessication than the open technique. The creams are spread on fine meshgauze, applied on the wounds, then covered with bulky protective gauze dressing and an elastic compressive wrap.

HEPARIN SALINE DRESSING

Heparin, a highly sulfated glycosoaminoglycan was studied originally of its anticoagulant property has been dealt in new prospective as an anti-inflammatory agent[62]. Nevertheless that heparin facilitates wound healing and shortens the recovery period in thermal burns was established already in guinea pigs and in clinical trials with human burns[62,63].

Heparin is postulated to play a positive role in angiogenesis or neovascularization and this process of formation of new blood vessels is an important step in wound healing. Heparin is proposed to be an anti inflammatory agent[64].

The pathogenesis of trauma in burns and the role of pro-inflammatory and inflammatory modulators in sepsis and trauma remain unclear. A better understanding of the modulation of interleukin-6 expression will enable us to develop more appropriate therapy for burn injury[65].

Efficacy of standard heparin on superficial partial thickness and deep partial thickness burn injury of 15 patients was assessed and compared with 15 control patients. Based on Total Burn Surface Area (TBSA), 15 patients were categorized into 3 groups and the modulation of serum interleukin-6 levels were assessed and compared with untreated control. Significant differences in cytokine IL-6 levels ($p<0.05$) were observed in heparin treated patients compared with untreated burn patients. There was significant variations in serum IL-6 on the day of admission and post burn day 10 in treated as well as untreated burn patients. Patients with higher percentage of total body surface area showed significant increase in IL-6 in the serum. The histochemical studies of the biopsy samples revealed that heparin treatment accelerated healing and reduced the time for remodeling. Clinical assessment of healing showed good correlation with histochemical and biochemical results. The administered heparin accelerated wound healing in burn patients[66].

The inflammation increase in size with swelling or edema in the local burn area of control groups and heparin treated burn patient swelling did not appear, or when it was present it subsided and did not reappear. Heparin treated burns in humans[67-73]. became progressively smaller in size was observed in the guinea pigs[62].

The beneficial effects were evident in thermal burns of humans when Saliba & Griner used large dose of heparin administrated both parenterally and topically[62,74]. Bacterial translocation [BT] continues to be a serious problem after severe burn, and is an important cause of mortality and morbidity[103]. Bacterial translocation is one of the most important causes of sepsis after severe burn injury[104]. Research in many experimental burn models has demonstrated that heparin increases survival and decreases BT[105,106]; however, it is still not known how heparin reduces BT, that is, which mechanism is responsible for these effects[64]. Recent work has demonstrated that heparin exerts anti-inflammatory effects on burns in humans and animals by inhibiting C1 esterase, an important enzyme in the complement cascade system[79,80]. This inhibitory action of heparin can be considered an immunosuppressive agent[27.49]

BIOLOGICAL DRESSING

All topical antimicrobial agents adversely affect wound healing, alter metabolic rate, and require re-application and daily maintenance. Biological dressing have no direct toxins of antimicrobial properties. However, they create a wound environment that prevents dessication,

diminishes bacterial proliferation, reduces loss of heat, water, protein and red blood cells, and promotes more rapid wound healing. Biological dressings also reduce burn wound pain[61].

These coverings include allograft (cadaver skin), xenograft (pig skin), Transcyte, Biobrane, and Integra. These should generally be applied within 24 hours of the burn injury, before high bacterial colonization of the wound occurs. Most often, synthetic and biological dressings are used to cover second-degree wounds while the underlying epithelium heals or it is used to cover full-thickness wounds for which autograft is not yet available. Each type of dressing has its advantages and disadvantages.

Biobrane consists of silicone and collagen manufactured into a sheet. This is placed on the wound and becomes adherent in 24 to 48 hours with dried exudates. This sheet then provides a barrier to moisture loss, and it provides a relatively painless wound bed that does not require dressing changes[75].

Biobrane should be used primarily in superficial second degree burn and split-thickness skin graft donor sites.

On the basis of work carried out in Lazovic G et al (2005)[53]., the following properties of collagen sheet are summarized.

Biological

- Collagen sheet is non-inflammatory
- Collagen sheet facilitates migration of fibro-blasts and microvascular cells
- Collagen sheet helps in the synthesis of neodermal collagen matrices
- Collagen sheet has low antigencity
- Collagen sheet has minimal biodegradation
- Collagen sheet is non - toxic
- Collagen sheet helps in minimizing scarring

Physiological

- Collagen sheet is impermeable to bacterial migration
- Collagen sheet modulates fluid flux from the wound
- Collagen sheet is elastic, soft, and supple
- Collagen sheet has good tear strength
- Collagen sheet has good suturing characteristics
- Collagen sheet has enough strength to be peeled off the wound

Adverse Effects

Collagen sheet has been found to be well tolerated in clinical trials. There have been no reports of clinically significant immunological or histological responses to the implementation of collagen sheet, and no reports of rejection of collagen sheet.

EXCISION AND GRAFTING

Deep second and third-degree burns do not heal in a timely fashion without autografting. In fact, the practice of leaving these dead tissues only serves as a nidus for inflammation and infection that could lead to the patient's death. Early excision and grafting of these wounds is currently done by most burn surgeons since reports have shown benefit over serial debridement in terms of survival, blood loss, and length of hospitalization.[76,77,78].

After a burn wound has been excised, the wound must be covered. This covering is ideally the patient's own skin. Wounds covering 20 to 30% TBSA can usually be closed at one operation with autograft split-thickness skin taken from the patient's available donor sites. In these operations, the skin grafts are not meshed, or they are meshed with a narrow ratio (2:1 or less), to maximize cosmetic outcome.

AIMS OF THE STUDY

- To study the profile of aerobic bacteria in patients with 20-40% burns.
- To identify the factors that influence the outcome in these patients.
- To determine the type of dressing that contributed to better outcome.
- To study the antibiogram pattern of bacteria in the burns unit.
- To determine the prevalence of β lactamase producing strains.
- To study the factors that contribute to prevention of infection and mortality in these patients.

MATERIALS AND METHODS

PLACE OF STUDY

This is a cross sectional study done involving 100 patients both male and female admitted to the Burn Unit, Department of Plastic and Reconstructive Surgery, Kilpauk Medical College and Hospital, Chennai. The study was carried out at the Institute of Microbiology, Madras Medical College, Chennai.

PERIOD OF STUDY

July 2005 to January 2006

STUDY GROUP

100 patients both male and female of age group 1 year to 65 years with burns of total body surface area (TBSA) of 20 to 40% were included in the study. These patients had burns ranging from deep partial thickness to full thickness injury. All patients received Tetanus toxoid booster upon admission.

SPECIMENS

1 Wound swabs from burn area.

2 Swabs from intact skin in the nasal, axillary, inguinal and umbilical regions of the patients. [If the skin in the above areas was burned samples were considered as burn wounds]

3 Blood from peripheral vein.

4 Environmental sampling study

COLLECTION & TRANSPORT OF SPECIMENS

Sterile cotton swabs were prepared using non-absorbent cotton wool, **(Rabbo and Benjamin 1951)** swab sticks were introduced into test tubes and plugged and were sterilized in the hot air oven at 160°C for 1 hour.

Wound Swab

Swabs were taken at the time of admission, 3rd or 4th day and 7th or 8th day. Swabs were taken from clinically deep areas of burn wound after cleansing.

Swabs from nasal, axillary, inguinal and umbilical regions were taken on the day of admission to study about the endogenous source of organisms. Swabs moistened in sterile saline were used to obtain nasal and skin samples.

BLOOD

Blood was obtained by aseptic venipuncture. For venipuncture, skin over the selected area for blood sampling is prepared by first cleaning with 70% alcohol and then with 2% Povidone Iodine. The disinfectant agent was allowed to act for 1 minute. In case of adults, about 5ml was collected. In case of infants and children only 3ml was

collected. Blood was directly inoculated into 50ml of Brain Heart Infusion broth.

ENVIRONMENTAL SAMPLES

Environmental sampling study was carried out from inanimate environment and the nursing staffs to find out the source of infection.

Swabs premoistened with normal saline were taken from the various sources of the environment & subjected to aerobic culture and other standard procedure for isolation and identification of microbial organisms.

The following were included as sampling sites.

1) Walls & Floors
2) McIntosh sheet
3) Linen
4) Cot
5) Dressing trolleys
6) Dressing Bin
7) Gloves
8) Antiseptic solutions
9) Tap water
10) Ward attender (male) I&II – nasal, throat and hand swabs
11) Ward attender (female) I&II – nasal, throat and hand swabs
12) Washing Room
13) Bath Room

Air contamination was studied by Blood agar settle plate method. A blood agar plate was kept near the ICU, male & female wards exposed for the duration of one hour. The plates were incubated and subjected to standard bacteriological studies.

The samples were transported immediately to the laboratory and processed.

MICROBIOLOGICAL ANALYSIS

DIRECT GRAM STAIN

Direct Gram stain smear of all the samples were done

CULTURE

Specimens were cultured on Nutrient agar, 5% sheep Blood agar and MacConkey's agar plates. Incubation was done at 37° C for 24-48 hours. The plates were examined for the growth at 24 hours. If growth was observed colony morphology and gram stain morphology were studied. If no growth was observed, the plates were further incubated for the next 48 hours. Blood agar was used to identify the gram positive cocci along with their haemolytic properties. MacConkey's agar was used to identify gram negative organisms, mainly the lactose fermenters & non-lactose fermenters. The bacterial isolates were then identified by means of Gram staining, motility and various biochemical reactions like Catalase, Oxidase, Indole, Citrate, Urease, Slide and Tube Coagulase, Triple sugar iron agar, Phenylalanine deaminase, Modified Hugh and Leifsons O/F test, Mannitol motility, Nitrate reduction, Methyl red,

Voges-proskauer, fermentation of sugars like Glucose, Sucrose, Lactose, Maltose, Mannitol, Mannose by standard microbiological techniques as recommended by NCCLS guidelines.

To perform the above Biochemical reactions the following media were used.

1. Peptone water.
2. Sugars-Glucose, Lactose, Maltose, Mannitol and Sucrose.
3. Glucose phosphate broth
4. Simmons citrate medium.
5. Christensen's urea agar.
6. Triple Sugar Iron Agar medium.

PROCESSING OF BLOOD

Brian heart infusion broth was used as the primary culture medium for blood and subcultures were made onto BAP, MAC and NA plate after 48 hours or on signs of growth like turbidity, lysis, pellicle and clot formation. The plates were examined for growth. The colony was then identified by Gram stain and various biochemical reactions and tested for its susceptibility to various antimicrobial agents. Subculture were made every third day for a period of 10 days and negative report was given if there was no growth.

PROCESSING OF SWABS – NASAL, AXILLARY, INGUINAL AND UMBILICUS

The swabs were inoculated onto Blood Agar Plate and MacConkey's Agar Plate and incubated at 37°C aerobically. The presence of growth was observed at 24 hours and 48 hours. If growth was present colonies were isolated and identification of the organism was done as described earlier.

DETECTION OF ANTIMICROBIAL SUSCEPTIBILITY PATTERNS

Antimicrobial susceptibility pattern was done on Mueller-Hinton agar by Kirby-Bauer Disc diffusion method as recommended by NCCLS.

Test inoculum-0.5 Mc Farland lawn culture.

Incubation-37°C for 16-18 hrs in ambient air.

Control strains:

Staph aureus-ATCC 25923.

E. coli-ATCC-25922.

Pseudomonas aeroginosa-ATCC 27853.

The antibiotics used [Discs were procured from **Himedia**]

Antimicrobial Agent	Inhibition zone in mm		
	Resistant	**Intermediate**	**Sensitive**
PenicillinG (10 U)	<20	21-29	>30
Ampicillin(10 mg)	<20	21-29	>30
Oxacillin(I mcg)			>13
Gentamicin(10 mcg)	>12	13-14	>15
Amikacin(30 mcg)	>14	15-16	>17
Ciprofloxacin(5 mcg)	>15	16-20	>21
Ofloxacin(5 mcg)	>12	13-15	>16
Cefotaxime (30mcg)	Gm+ve<14 Gm-ve<10	15-22 11-15	>23 >16
Ceftazidime(30 mcg)	14	15-17	>18
Imipenem (10mcg)	<13	14-15	>16
Vancomycin(30 mcg)			>15

The diameters of the zones of inhibition were interpreted according to NCCLS standards for each organism.

IDENTIFICATION OF BETA-LACTMASE PRODUCTION:

The determination of beta-lactamases production was done by iodometric method.

PROCEDURE

IODOMETRIC METHOD

REQUIREMENTS

i) 1% soluble starch solution prepared by dissolving the starch at 100°C

ii) Iodine reagent consisting of 2.03g iodine and 5.32 g potassium iodide in 100 ml distilled water.

iii) Microtitre plate.

PROCEDURE

From an overnight incubation culture of the test organism, a heavy suspension was made (containing 10^9 CFU/ml) in 100mM sodium phosphate buffer at pH7.3 containing penicillin at 6g/litre, along with positive and negative controls.

Positive control – E. coli ATCC 35218.

Negative control – E. coli ATCC 25922.

The test and the control organisms were inoculated into the wells of a microtitre plate. After incubation for 1 hr at 37°C, two drops of freshly prepared 1 % soluble starch solution were added to each well. A drop of iodine reagent was then added. If blue colour was lost within 10 minutes, the presence of beta lactamase was inferred. If, however, the blue persisted, the culture was considered to be beta-lactame-negative.

DETECTION OF EXTENDED-SPCTRUM BETA-LACTAMASES (ESBL)

ESBLs are enzymes that meditate resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect cephamycins (e.g., cefoxitin).

Cefpodoxime and ceftazidime have been proposed as indicators of ESBL production.

METHODS ADOPTED FOR DETECTION

Isolates showing a zone of inhibition<22 mm for ceftazidime were tested for ESBL production as per NCCLS criteria, using standard control strains.

1. COMBINED DISC METHOD

Latest guidelines by NCCLS recommend a combined disc method as an indicator of ESBL production. For disc diffusion testing, ≥5 mm increase in zone diameter of the antimicrobial agent tested in combination with betalactamase inhibitor (e.g., clavulanic acid, sulbactam) versus its zone when tested alone confirms an ESBL producing organism.

Combined Disc method using cefaperazone (75 mcg) and cefaperazone-sulbactam (75 mcg/30 mcg) was performed for phenotypic confirmation of ESBL production.

2. DOUBLE DISC SYNERGY TEST

In this test a lawn of the test strain on Mueller Hinton agar was exposed to discs of ceftazidime (30 mcg) and augmentin (20 mcg amoxycillin/10 mcg clavulanic acid) arranged in pairs. The discs were arranged so that the distance between them is approximately twice the radius of the inhibition zone produced by the cephalosporin tested on its own. After overnight incubation, the test strain was an ESBL producer if the inhibition zone around the cephalosporin disc was extended on the side nearest the augmentin disc.

SCREENING FOR Amp-C AND ESBL PRODUCING ISOLATES USING MODIFIED DOUBLE DISK SYNERGY TEST[107]

Isolates with zone of inhibition <22 mm for ceftazidime were tested. A 0.5 McFarland turbidity of test isolate was swabbed on Mueller Hinton agar plates and disk of ceftazidime (30µg) was placed adjacent to clavulanic acid in (10 µg) and cefoxitin (30µg) disc was placed at a distance of 20 mm from each other. After incubation an enhanced zone of inhibition between ceftazidime and clavulanic acid were interpreted as presumptive evidence for the presence of ESBL.

Isolates that showed reduced susceptibilty ceftazidime and cefoxitin were considered as screen positive and selected for detection of Amp C b-lactamases.

CONFIRMATORY TEST FOR Amp C BETA LACTAMASES

Amp C DISC TEST

A lawn culture of E.coli ATCC 25922 was prepared on MHA plate. Sterile discs (6mm) were moistened with sterile saline(20µl) and inoculated with several colonies of test organism. The inoculated disc was then placed on the inoculated plate beside a cefoxitin disc almost touching it. The plates were incubated overnight at 35ºC .A positive test appeared as flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disc. A negative test had an undistorted zone.

MINIMUM INHIBITORY CONCENTRATION (MIC)

MIC was done for the drug cefotaxime by agar dilution method for Gram negative bacilli.

PROCEDURE

MEDIA

19 ml of Mueller-Hinton agar is prepared in tubes, autoclaved and allowed to cool in a 50°C water bath.

ANTIBIOTIC

i) Cefotaxime was dissolved in sterile water. Then serial dilutions of the antibiotic from 1mg/l to 256mg/l were then made. One ml of the antibiotic solution was added to 19 ml of MHA and poured into the petri dish. A control plate containing the test medium without the antibiotic is prepared for each series of the dilution. After the plates have set, they were dried at 37°C for 20 minutes in the incubator.

TEST INOCULUM

The test strains were inoculated in peptone water and its turbidity adjusted to 0.5 McFarland standard to get a working inoculum of 10 CFU/ml. A multipoint micropipette was used to deliver 2 µl so that the final inoculum on the agar surface would be 10 CFU/spot.

CONTROLS

Standard control strains were used in every batch of MIC tests.

ENVIRONMENTAL STUDY

1. Swabs premoistened with normal saline taken from various parts were processed using standard microbiological methods as described earlier.

2. Water sampling[108] - 100ml of water was inoculated into equal volume of double strength MacConkey broth and inoculated at 37°C for 48 hours. Then subculturing was done on Blood Agar Plate, MacConkey's Agar Plate and processed using the standard microbiological methods.

3. Air contamination was looked for in the intensive care unit, male and female wards. This was done by exposing Blood Agar Plate for duration of 1 hour. The plates were incubated at 37°C aerobically for 24 hours and then processed. The colony count was done and the bacteria was identified as described earlier. The antibiogram was done for the bacteria isolated from the environmental study.

RESULTS

Table 1: INCIDENCE OF AGE AND SEX IN THE BURNS CASES

Age Group In Years	Male	Female	Total
1-10	6	3	9
11-20	8	14	22
21-30	14	22	36
31-40	13	10	23
41-50	1	4	5
51-60	-	1	1
61-70	2	2	4
Total	44	56	100

Young females were found to be most commonly affected.(11-40yrs) Of the 9 Paediatric patients 6 were less then 6 years old.

Table 2: CAUSES OF BURNS

		Children		Adults	
		Male	Female	Male	Female
Flame	81	-	-	35	46
Scald	17	6	3	3	5
Electrical	2	-	-	2	-

Flame was the most common cause (81%) in this study followed by scalds. The electrical injury was the least common cause and observed in 2 cases. Scald injuries were observed more commonly in children and women.

Table: 3. PERCENTAGE OF BURNS IN RELATION TO AGE & SEX

	20 – 30 %		Total	31 – 40 %		Total
	Male	Female		Male	Female	
0-12	4	2	6	2	1	3
13-35	21	26	47	11	19	30
36-60	4	4	8	-	2	2
>60	2	1	3	-	1	1
Total	31	33	64	13	23	36

Among 100 patients 64 cases presented with 20-30% Burns. More no. of 20-30% burns have been observed in young adults.

Table 4: MORTALITY RATE IN RELATION TO AGE

Ages In Years	Total 100	Mortality 41	Percentage 41%
Children 0-12	9	2	22.2%
Adults 13-35	77	32	41.5%
36-60	10	6	60%
>60	4	1	25%
Total Adults	91	39	42.85%

Table 5:

CORRELATION OF MORTALITY WITH DEGREE OF BURNS

	II° Superficial No: 20		II° Deep No: 27		III° No: 3		Mixed			
							I&II° No: 28		II&III° No: 22	
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**
Survival	18		21		-		15		5	
Death	2	**10**	6	**22**	3	**100**	13	**46**	17	**77**
Total	20		27		3		28		22	

A higher mortality rate was observed in patients with III° and II°&III° mixed burns.

Table 6

MORTALITY AND SURVIVAL IN TERMS OF TIME DURATION

	< 1 week	**1-2 weeks**	**2-3 weeks**	**3-4 weeks**	**> 4 weeks**
No. of death 41	32 (78%)	4 (9.75%)	2 (4.87%)	2 (4.87%)	1 (2.43%)
No. of discharges among survivors 59	26 (44.06%)	18 (30.50%)	3 (5.08%)	7 (11.86%)	5 (8.47%)

Highest percentage of mortality occurred within the first week. Among the survivors most were discharged within the first 2 weeks of hospitalization.

Table 7: CORRELATION BETWEEN MORTALITY AND PERCENTAGE OF BURNS

% of Burns	Children No: 9				Adults No: 91			
	Male		Female		Male		Female	
	No. Of Patients	Death	No. Of Patients	Death	No. Of Patients	Death	No. Of Patients	Death
20-30%	4	-	2	-	27	5 **19%**	31	8 **26%**
31-40%	2	1 **50%**	1	1 **100%**	11	6 **54%**	22	20 **91%**
Total	6	1	3	1	38	11	53	28

The mortality of females with 31-40% of Total Burn Surface Area was the highest.

Table: 8 DEPTH OF BURN & TYPE OF DRESSING

	Type Of Dressing					
	Collagen		Heparin Saline		SSD	
	Children	Adults	Children	Adults	Children	Adults
II° Superficial	4	3	-	13	-	-
II° Deep	2	3	-	22	-	-
III°	-	-	-	2	-	1
Mixed						
I& II	3	9	-	14	-	2
II&III	-	-	-	18	-	4
Total	9	15	-	69	-	7

Heparin saline dressing was commonly applied followed by collagen dressing. In children only collagen dressing was applied.

Table: 9: TYPE OF DRESSING AND MORTALITY

	Collagen Total 24		Heparin Saline Total 69		SSD Total 7	
	No	%	No	%	No	%
Survival	18	75	38	55	3	43
Death	6	25	31	45	4	57

Mortality was reported more in dressings with Silver Sulpha Diazine followed by Heparin saline. Collagen dressing had the best outcome.

Table: 10 MORTALITY IN TERMS OF TIME DURATION IN INHALATION INJURY

Patients with inhalation injury	Death	
	No of Cases	%
8 (9.8%)	8	100%

Among 81 flame injury patients 8 patients had inhalation injury. 100% mortality occurred in burns with inhalation injury.

Table 11: SOURCE OF INFECTION - ENDOGENOUS

Organisms	Nose Swabs No. 73	Axillae Swabs No. 82	Umbilicus Swabs No. 67	Groins Swabs No. 84
CONS	14	10	3	
S. aureus	7		1	
Micrococci		17	10	6
Diphtheroids		8	10	7
E.coli				5
Klebsiella spp.				13

Table 12: SOURCE OF INFECTION - EXOGENOUS

Organisms	Inanimate ICU, Male & Female Wards					Ward Attenders
	Floor and Walls	Linen	Cot	Trolley	Blood Agar settle plate	
Ps. aeruginosa	2	2	1	1	3	
Acinetobacter spp	3	2		1	3	
E. coli	1					
Klebsiella spp		4			3	
Diphtheroids	2					2
S. aureus					1	2
CONS						4

37 isolates were obtained from hospital environment & staffs

Table: 13 PATHOGENIC ORGANISMS ISOLATED

	Day 1 I Swab No: 100	%	Day 3&4 II Swab No: 94	%	Day 7 III Swab No: 43	%
No Of Swabs	100		94		43	
No Growth	66	66%	-	-	-	-
Monomicrobial	30	30%	42	44.6%	11	25.5%
Polymicrobial	4	4%	52	55.31%	32	74.41%

On admission polymicrobial type of infection was less and it was more with the patients who stayed in the hospital for longer days.

Table: 14 PATHOGENIC ORGANISMS ISOLATED IN WOUND SWABS

Organisms Isolated	I Swab No.100 Organisms No. 38	II Swab No. 94 Organisms No. 166	III Swab No. 43 Organisms No. 89
Monomicrobial	**30**	**42**	**11**
Pseudomonas aeruginosa	7	20	6
Klebsiella spp	4	13	3
Acinetobacter spp	2	1	
Escherichia coli		4	
CONS	12		
Staphylococcus aureus	5	4	2
Polymicrobial	**4**	**52**	**32**
Pseudomonas + *Acinetobacter*		6	8
Pseudomonas + *Escherichia coli*		3	
Acinetobacter+ *Klebsiella*		11	3
Klebsiella+ CONS	2		
Pseudomonas + *Klebsiella*		12	7
Pseudomonas + CONS	2		
Pseudomonas + *Acinetobacter*+ *Klebsiella*		11	8
Pseudomonas + *Acinetobacter*+ S. aureus		5	4
Pseudomonas + S. aureus+ *Klebsiella*		4	2

The initial swabs were predominantly monomicrobial with gram positive isolates and which is replaced by gram negative isolates in the later

swabs, which were also polymicrobial

Table 15 BACTERIAL ISOLATES AT VARIOUS LEVELS

ORGANISMS	I SWAB (100) - ORGANISMS No. 38						II SWAB (94) - ORGANISMS No. 166						III SWAB (43) - ORGANISMS No. 89					
	Single		Mixed		Total		Single		Mixed		Total		Single		Mixed		Total	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
Gram negative bacilli						44.64						92.16						91.02
Pseudomonas aeruginosa	7	18.42	2	5.26	9	23.68	20	12.04	41	24.69	61	36.73	6	6.74	29	32.58	35	39.32
Klebsiella spp	4	10.52	2	5.26	6	15.78	13	7.83	38	22.89	51	30.72	3	3.37	20	22.47	23	25.84
Acinetobacter spp	2	5.26			2	5.26	1	0.6	33	19.87	34	20.47			23	25.84	23	25.84
Escherichia coli	-						4	2.4	3	1.8	7	4.2						
Gram positive cocci						55.25						7.82						8.98
Staphylococcus aureus	5	13.15			5	13.15	4	2.4	9	5.42	13	7.82	2	2.24	6	6.74	8	8.98
CONS	12	31.57	4	10.52	16	42.1												

Table: 16 PATHOGENIC ORGANISMS

ORGANISMS	TOTAL No:293	PERCENTAGE
Pseudomonas aeruginosa	105	35.84
Klebsiella spp	80	27.30
Acinetobacter spp	59	20.13
Escherichia coli	7	2.38
Staphylococcus aureus	26	8.87
CONS	16	5.46

Pseudomonas aeruginosa was the commonest organism isolated. Gram Positive cocci found only 14.33% of the total isolates.

Table: 17 BLOOD CULTURE REPORT

	Positive Cultures	**Negative Cultures**
Total No. Of Blood Cultures 94	21 (22.34%)	73 (77.65%)
No. Of Survivors	2 (9.6%)	51 (69.86%)
No. Of Death	19 (90.4%)	22 (30.13%)

The mortality among blood culture negative patients could have been due to other factors, or due to commencement of antibiotic therapy.

Table: 18 PATHOGENIC ORGANISMS ISOLATED FROM BLOOD CULTURE

	Ps. aeruginosa	***Acinetobacter***	***Klebsiella***
Blood Culture positive with Death No:19	18 (94.7%)		1 (100%)
Blood Culture positive with Survival No:2	1	1	

All 21 organisms isolated from the Blood culture were similar to the organisms isolated from the wound swabs.

Table :19 ANTIBIOTIC SENSITIVITY PATTERN OF GRAM NEGATIVE BACILLI

organisms	No. of Organisms	Ciprofloxacin	Ofloxacin	Amikacin	Gentamycin	Ampicillin	Cefo - taxime	Ceftazidime	Cefaperazone	Cefaperazone Sulbactam	Imipenem
Pseudomonas aeruginosa	105	64 61%	70 67%	82 78%	78 74%	62 59%	78 74%	82 78%	87 83%	105 100%	105 100%
Klebsiella spp	80	69 86%	54 68%	59 74%	52 65%	60 75%	66 83%	66 83%	68 85%	80 100%	80 100%
Acinetobacter spp	59	43 73%	40 68%	52 88%	33 56%	43 73%	30 51%	46 78%	49 83%	59 100%	59 100%
Escherichia coli	7	3 43%	2 29%	3 43%	2 29%	3 43%	2 29%	2 29%	4 57%	7 100%	7 100%

Table: 20 ANTIBIOTIC SENSITIVITY PATTERN OF GRAM POSITIVE COCCI

Organisms	No. of Organisms	Penicillin	Ampicillin	Cloxacillin	Oxacillin	Ofloxacin	Ceftazidime	Cefotaxime	Gentamicin	Vancomycin
S. aureus	26	6 23%	12 46%	13 50%	18 69%	21 81%	20 77%	20 77%	12 46%	26 100%

CONS	16	8 50%	6 38%	10 63%	12 75%	11 69%	12 75%	13 81%	7 44%	16 100%

Table: 21 β – LACTAMASE PRODUCTION

Organisms	No of β lactamase producers	Percentage
S. aureus No: 26	16	62%
CONS No: 16	9	56%

Table: 22 ESBL PRODUCERS

Organisms	No of ESBL producers	Percentage
E coli (7)	4	57.14%
Klebsiella spp (80)	11	13.75%
Ps. aeruginosa (105)	10	9.52%

Ceftazidime resistant isolates – E.coli (5), Klebsiella (14) and Ps.aeruginosa (23) were tested for ESBL production.

Table: 23 MIC OF CEFOTAXIME FOR GRAM NEGATIVE ISOLATES

Organisms	16 mg/l	64 mg/l	125 mg/l	250 mg/l	>250 mg/l
P. aeruginosa - 27		4	2	9	12
Klebsiella spp - 14	4	3	3		4
E coli - 5		2		2	1

4 Klebsiella isolates showed intermediate sensitivity to cefotaxime on Kirby-Bauer disc diffusion method.

DISCUSSION

Burns provide a suitable site for bacterial multiplication and infection, mainly because of the larger area involved and longer duration of patient stay in the hospital. To ensure early and appropriate therapy in burn patients, a frequent evaluation of the wound is necessary. Therefore, a continuous surveillance of microorganisms and a regular update of their antibiotic resistance pattern is essential to maintain good infection control programmes in the burn unit, thus improving the overall infection-related morbidity and mortality.

The age and gender distribution [table 1] shows that the incidence was more in young adults between 11 – 40 years of age. Jayaraman et.al[83] study showed 58.9% of burns occurred in young adults between 11-30 years of age. Males exceeded females upto 10 years of age. Burn occurred more frequently in young females belonging to the age group of 13-35 years out of 56 total females (80.35%) [table 3]. This may be due to clothing catching fire while cooking on a floor level kerosene stove[84]. This study corresponds with the study made by V. Jayaraman et.al (1993)[83]. In their study the incidence of females were 55.8% and the females belonging to age group of 13-36 years had higher incidence.

The most common cause of injury (table 2) was flame 81%. Scalds come next to flame burns. Herndon et.al(2001)[8] stated in children the most common burns are scalds. In our study scald injuries were observed more commonly in children and women [table 2]. This

study corresponds with other studies of Jayaraman et.al (1993)[84] (26.8%) and Vaishnav at.al (1974)[85] 29%. In our study electrical burns were few 2%, Steven E. Wolf[86] quoted 3 to 5% of all admitted patients of injured from electrical contact.

In this study patients with burns of 20-40% were included. More than 40% and less then 20% were not studied since patients with more than 40% burns had been observed to have a high mortality rate and most of the burns of less than 20% healed without infection. Jayaraman et.al., in their work on Epidemiology of Burn injury quoted the possibility of survival was present only in patients having burn less than 50% Total Burn Surface Area.

Table 3 shows 64% were between the range of 20-30% and only 36% were between the range of 31-40%.

The total mortality rate was 41% and the mortality rate for children was 22.2% and 42.85% for adults (table 4). Jayaraman et.al reported 40.6% of total mortality rate. Among the total no. of deaths, more deaths occurred in females [table 7]. This corresponds to the study of Mathangi et.al[90] who had stated 67% of total no. of patients who had died were females between 13-35 years of age. In this study 78% of the death occurred within a week's time and 9.75% of death occurred in the 2nd week (table 6). Mathangi et.al reported 91% of death occurred within 5days. Among the 9 children, death occurred in 2 children. The percentage of burns in these 2 children were between 31-40% (table 7).

Heparin saline dressing was most commonly applied 69% (table 8). Next commonly used dressing was collagen dressing 24%. Collagen dressing was applied in both children and adults. For children only collagen dressing was applied.Collagen dressing was used only for II° superficial & deep and I & II° mixed burns. For III° burns and II & III° mixed burns closed dressing with heparin saline /SSD were applied. (table 8) The mortality rate is more in patients with Silver Sulpha Diazine 57% and heparin saline dressing 45%.(table 9)

Jayaraman et.al stated 28% of patients had inhalation injury. In our study 9.8% of patients had inhalation injury(table 10). Schwartz[81] stated the presence of inhalation injury strongly correlates with infection and Wolf et.al[82] stated inhalation injury increases the mortality rate. In our study mortality rate with smoke inhalation injury was 100%. Death occurred within 1 week time. The mortality of burn patients in burn units is well recognized to be related to the age of the patients (table 4) and the percentage of total area of burn wound[27] (table 7) and the presence or absence of concomitant inhalation injury (table 10). Tredget et.al and Ryan et.al also stated the similar findings. In this study mortality is more with III° and II° & III° mixed burns (table 5).

In this study length of stay in hospital for those who survived was less than 1 week for 44.0%, and 30.50% were discharged on 2nd week (table 6).Mathangi et.al quoted 52.8% and 28% patients were discharged on the 1st and 2nd week respectively

In this study the pattern of burn wound microbial colonization was evaluated. The time related changes in the predominant flora was also evaluated throughout the patients hospital stay. Wound swabs were taken on different occasions (tables 13 and 14) the detailed bacteriological study was undertaken from 100 patients with 20-40% burns.

Total no. of wound swab sampling on admission were 100 (day 1) and as 6 burns patients died within 4 days, II wound swab sampling (day 3&4) were done for 94 patients.

III wound swab sampling were done on 7^{th} or 8^{th} day. 43 sampling were done as the total no. of death within one week were 32 and 25 patients were discharged.

Nasser et.al (2003)[46] study shows that on admission no mixed growth occurred in cultures. There was predominance of gram positive isolates (70.7%) and gram negative isolates accounted for 27.6%. In our study on admission, 55.25% were gram positive isolates and 44.64% were gram negative isolates and mixed growth occurred in 4%(tables 13, 15).

During the first 4 days (II swab) Nasser et.al study showed the prevalence of gram negative isolates and less prevalence of gram positive isolate. In our study 94 sampling procedure were performed and 166 isolates were obtained (table 14). Among these total isolates 92.16% were gram negative bacilli and only 7.82% were gram positive isolates (table 15) with 55.31% showing mixed growth.(table13)

After 7 days 43 sampling procedures were done and mixed growth occurred in 74.41%, yielding 89 isolates (tables13&14). Among these total isolates, 91.02% were gram negative bacilli and 8.98% were gram positive cocci (table 15).

Lari et.al (2000)[91] study showed a predominance of gram positive isolates on admission, which was replaced by gram negative isolates, subsequently. Various studies (Manson et.al (1992)[87], Pruitt et.al (1984)[88] Wurtz et.al (1995)[89]) also showed that the gram-positive organisms are gradually replaced by gram-negative organisms.

Agnihotri et.al (2004)[51] in their study stated major aerobic bacterial isolates from wound swabs were *Pseudomonas aeruginosa* 59%, *Staphylococcus aureus* 17.9%, *Acinetobacter* species 7.2% and *Klebsiella* species 3.9%.

In our study the predominant organisms isolated (table 16) were *Pseudomonas aeruginosa* [35.84%], *Klebsiella* species [27.30%], *Acinetobacter* species [20.13%], *Escherichia coli* [2.38%] *Staphylococcus aureus* [8.87%] and CONS [5.46%].

In this study swabs I [4%], swabs II [55.31%], swabs III [74.41%](Table 13) showed presence of polymicrobial infection. The most common combination was *Pseudomonas* aeruginosa with *Klebsiella* species or *Acinetobacter* species or both. This finding is in accordance with the study of S. P. Thyagarajan and K. Mathangi Ramakrishnan (1987-1989)[90] who also found mixed infection in burn cases.

Our findings concerning the high frequency of *Pseudomonas aeruginosa* (35.84%) (table 16) greatly coincide with many previous reports where this organism was held responsible for majority of burn wound infections.(Lari et.al (2000)[91], Cremer et.al (1996)[102].

Ozumba et.al (2000)[92] study state that *Klebsiella* isolates were more predominant than *Pseudomonas* . But in our study *Klebsiella* was the second most frequently recovered organisms [27.30%] (table 16).

Various studies of Kaushik et.al (2001)[47], Sengupta et.al (2001)[48], Ananthakrishnan et.al (2002)[50] had shown that *Acinetobacter* species are rapidly emerging as important pathogens predominantly in burns units. Our study showed *Acinetobacter* species (20.13%) (table 16) were the third predominant organisms isolated and most of them isolated from mixed infections.

Staphylococcus aureus came fourth in the list of microbial isolates recovered in our study [8.87%] (table 16). This is contrary to many previous reports including the study of K. Mathangi et.al and S. P. Thyagarajan et.al and Singh et.al (2003) which indicated a much higher frequency of isolation of this organism.

Nasser et.al study revealed CONS were recovered at a frequency of 11.6%. Vindenes et.al[93] study revealed higher rates of recovery of CONS in the burn wounds. In our study Coagulase Negative Staphylococci were recovered at a frequency of 5.46% (table 16), the organisms were isolated mainly from earlier wound swabs, taken on admission of the patients.

McManus et.al[43] in 1985 suggested that 10% of all burn patients develop *Pseudomonas* bacteremia which carries a mortality rate of 80% and the risk of *Pseudomonas* infection increases substantially in burns more than 30%. Lari et.al[91] in their study revealed the majority of bacteremic patients (most of them were *Pseudomonas* infections.) died (89%) which is higher than that reported by Flick et.al[94] and Kreger et.al [95].

In our study 21 patients (22.34%) developed bacteremia (Tables 17,18) of which 19 (90.4%) died. Among 21 patients *Pseudomonas was* isolated in 19 patients of whom 18 died (mortality rate of 94.7%) and bacteremia due to *Klebsiella* was observed in one patient who died, which caries mortality rate of 5.3%. One patient had *Acinetobacter* bacteremia, but responded to treatment.The mortality among blood culture negative patients could have been due to commencement of antibiotic therapy or due to other factors.

ANTIBIOTIC SUSCEPTIBILITY PATTERN

GRAM NEGATIVE BACILLI (Table 19)

The gram negative bacilli exhibited varying degree of sensitivity to the aminoglycosides from 29% to 88%, for fluroquinolones 29% to 86% for penicillins from 43% to 75% and to cephalosporins from 29% to 85%. All the isolates were 100% sensitive to cefaperazone sulbactam and imipenem.

Pseudomonas aeruginosa

Tredget et.al (2004)[97] in their study states that among the third generation cephalosporins only ceftazidime and cefaperazone have been found to have anti-*Pseudomonal* activity with ceftazidime being more active. Our study showed *Pseudomonas* resistance to ceftazidime (22%) and cefotaxime (26%).

Singh et.al (2003)[98] revealed the incidence of *Pseudomonas* resistance to ceftazidime 62% and cefotaxime 66%. Revathi et.al in their study revealed *Pseudomonas* resistance to ceftazidime 17% and cefotaxime 39%.

Gales et.al (2001)[96] in their study stated meropenem is active in upto 95% *Pseudomonal* isolates worldwide. But Tredget et.al states only 29% of isolates were susceptible to meropenem. In our study 100% of *Pseudomonal* isolates were sensitive to imipenem. (carbapenem).

Revathi et.al (1998)[35] study stated 27% of *Pseudomonas* were resistant to amikacin and 47% of *Pseudomonas* were resistant to gentamicin. Singh et.al stated 52% of *Pseudomonas* were resistant to amikacin and 69% were resistant to gentamicin. In our study among *Pseudomonal* isolates 22% were resistant to amikacin and 26% resistant to gentamicin.

Gales et.al stated that worldwide 60% of Latin America and 85% Asia-Pacific strains of *Pseudomonas* were sensitive to ciprofloxacin.

Tredget et.al stated only 54% *Pseudomonas* were susceptible. In our study 61% of *Pseudomonas* were susceptible to ciprofloxacin.

Gales et.al (2001) found that multi drug resistant *Pseudomonas* were 8.2%, while in our study 27% of *Pseudomonas* were multi drug resistant.

Altoparlak et.al (2005)[101] stated overall resistance rates of *Pseudomonas* were imipenem 30.8%, meropenem 32.5%, ceftazidime 72.5%, cefeperazone-sulbactam 52.5%, ofloxacin 66.7%, ciprofloxacin 60.8%, amikacin 14.2% and gentamicin 28.3%.

***Klebsiella* species**

Revathi et.al[35] and Singh et.al[98] stated 78% and 84% of *Klebsiella* isolates were resistant to cefotaxime respectively. In our study 17% of isolates were resistant to both cefotaxime and ceftazidime and 15% resistance with cefaperazone. 100% of *Klebsiella* isolates were sensitive to imipenem and cefaperazone-sulbactam. Both May et.al (2000)[99] and Landman et.al (1999)[100] have described their experience with an increase in multi-drug resistant *Klebsiella* with Cephalosporins. In our study 14% of multi-drug resistant *Klebsiella* were isolated.

***Acinetobacter* species**

Revathi et.al and Singh et.al stated 81.8% and 94% *Acinetobacter* were resistant to cefotaxime. In our study 49% of *Acinetobacter* were resistant to cefotaxime. 100% of isolates were sensitive to cefaperazone-sulbactam and imipenem. Altoparlak et.al

stated resistant rates of *Acinetobacter* as follows: imipenem 33.3%, meropenem 33.3%, ceftazidime 66.7%, cefaperazone-sulbactam 33.3%, ofloxacin 44.4%, ciprofloxacin 33.3%, amikacin 11.1% and gentamicin 22.2%.

Singh et.al stated 90% of *Acinetobacter* were multi-drug resistant which is much higher than the study conducted by NCCLS (National Committee for Clinical Laboratory Standards) and Sengupta et.al. In our study 42% of *Acinetobacter* were multi-drug resistant, though there was no resistance to cefaperazone-sulbactam and the carbapenems.

Escherichia coli

43% of *Escherichia coli* were sensitive to ciprofloxacin, amikacin and ampicillin. 29% of the isolates were sensitive to ofloxacin, gentamicin, cefotaxime and ceftazidime. 100% of the isolates were sensitive to cefaperazone-sulbactam and imipenem.

GRAM POSITIVE COCCI

Staphylococcus aureus was 100% sensitive to vancomycin. This corresponds with Singh et.al and Revathi et.al studies. Komolafe et.al 2003[54] stated 6.5% of *Staphylococcus aureus* was sensitive to ampicillin and 33.3% was sensitive to penicillin. In our study 46% was sensitive to ampicillin and 23% was sensitive to penicillin. Komolafe et.al 2003[54] stated the following sensitivity pattern of *Staphylococcus aureus*-penicillin 33.3%, ampicillin 6.5%, cloxacillin 20.4% and gentamicin 10%. In our study the following pattern of antibiotic sensitivity were observed-

penicillin 23%, ampicillin 46%, cloxacillin 50%, gentamicin 46% and ceftazidime 77%. Kaushik et.al quoted 45.8%, 32.6% and 33.3% of *Staphylococcus aureus* were sensitive to cloxacillin, gentamycin and ceftazidime respectively.

This study showed the following sensitivity pattern of Coagulase Negative *Staphylococcus*-ampicillin 38.5%, cloxacillin 63%, gentamicin 44%, penicillin 50% and vancomycin100%. Komolafe et.al showed only 25% of CONS were susceptible to ampicillin and gentamicin, 100% of the CONS were resistant to penicillin and cloxacillin.

Beta Lactamases

Beta lactamase production was observed in 56% of CONS and 62% of the isolates in *S.aureus(Table21).* Methicillin resistant *S.aureus was* 31% .

ESBL

In this study, ESBL producing strains were detected in 57.14% of *Escherichia coli* and 13.75% of *Klebsiella spp* .(Table 22) Though the methodology is not yet recommended for *Pseudomonas spp*, 9.52% of *Ps.aeruginosa* isolates showed ESBL production by double disc method. Amp C Beta lactamase production was not detected in this study. Singh et.al., in their study stated 89% of *Klebsiella spp* and 22% of *Pseudomonas spp* where ESBL producers.

MIC –Minimum Inhibitory Concentration

In this study minimum inhibitory concentration break points for cefotaxime resistant *Klebsiella spp* were between 16 mg/l to >250 mg/l and *Ps.aeruginosa* were between 64 mg/l to >250 mg/l.(Table 23) and *E.coli* were between 64mg/l to >250mg/l

Environmental sampling

Environmental sampling was done for the purpose of tracing the source of infection-endogenous (Table11) and exogenous (Table 12). The pathogenic organisms isolated from various sites on the day of admission were *S.aureus*(7) from nose, *E.coli*(5) and *Klebsiella spp*(13) from the groins. Among the 7 nasal carriers of *S.aureus*, 4 were found to be methicillin resistant.

The organisms isolated from the environmental and hospital staff were *Ps.aeruginosa, Acinetobacter spp, E.coli, Klebsiella spp* and *S.aureus*. None of the isolates showed methicillin resistant *S.aureus*. Environmental samples showed growth of bacteria with sensitivity pattern of all the isolates and pigment production of *Ps.aeruginosa* similar to those of isolates recovered from nearly 50% of the patients.

SUMMARY AND CONCLUSION

- 100 patients with 20-40% burns admitted to a tertiary care center were included in the study.
- Majority of the patients affected were 20-30 yrs of age.
- Children formed 9% of study group.
- There was a slight female preponderance (56%)
- The commonest cause of burns was flame injury, followed by scalds. Electrical injuries were least common.
- Patients with 20-30% of Total Burn Surface Area formed 64% of the study population.
- Inhalation injuries ,third degree burns and second degree and third degree mixed burns had a high mortality rate.
- Patients with collagen dressing had better outcome (75% survival) as compared to Heparin Saline dressing (55%) and Silver Sulfa Diazine (43%).
- 22.34% patients developed bacteremia . Among these 90.4% had a fatal outcome.
- Mortality rate was higher (60%) in the older age group (36-60 yrs).

- Colonisation of burn wound was observed in 34% of patients on the day of admission itself. It was predominantly monomicrobial with Gram positive cocci. Subsequent swabs showed 100% colonisation with a shift to polymicrobial infection with Gram negative bacilli predominating.

- *Ps.aeruginosa* was the commonest isolate (35.84%) and followed by *Klebsiella spp* (27.30%) and *Acinetobacter spp (*20.13%).

- Beta lactamase production was observed in almost 60% of all Gram positive cocci. Methicillin resistance was observed in 31% of *S.aureus* strains. No resistance to vancomycin was observed.

- Among Gram negative bacilli Multi Drug Resistant (MDR) strains ranged from 9.3% in *Klebsiella spp* to 27% among *Ps.aeruginosa* ,42% in *Acinetobacter isolates and* 60% of *E.coli* .

- High degree of resistance to third generation cephalosporins with MIC levels more than 256 mg/l in cefotaxime was seen in 16 of these isolates.

- ESBL production was detected in 9.52% of *Ps.aeruginosa,* 13.75% of *Klebsiella spp* and 80% of *E.coli*.

- No metallo betalactamase activity was detected

- Environmental samples showed growth of bacteria with sensitivity pattern and pigment production similar to that of isolates from nearly 50% of the patients.

This suggests the need for surveillance of environmental saprophytes, monitoring for MDR strains in the environment so as to identify and block the transmission of these strains to the patients.

Measures such as the following have to be implemented with appropriate supervision to evidence, that there is no break in the aseptic techniques.

1. To reduce the nosocomial infections, improved barrier nursing, personal hygiene and restriction of visitors should be strictly followed.
2. Discarded dressing materials should be properly disposed without delay.
3. Use of sterile disposable aprons or separate sets of autoclaved linen for each dressing should be provided.
4. Adequate spacing of beds is necessary.
5. Periodical fumigation of all the wards of burns unit should be under taken.
6. **Necessary equipments to provide respiratory support is needed in patients with inhalation injury.**
7. As the outcome of collagen dressing was good, patients may be provided with collagen dressing as per the clinical indication eventhough it is costly.

8. Routine surveillance of the environment with rotation of antibiotics, based upon the susceptibility pattern of environmental isolates can also reduce the incidence of nosocomial infection.

Table :19 Antibiotic sensitivity pattern of gram negative bacilli

ORGANISMS	No. of Organisms	Ciprofloxin	Ofloxacin	Amikacin	Gentamycin	Ampicillin	Cefo - taxime	Cef zidi
Pseudomonas aeruginosa	105	64 61%	70 67%	82 78%	78 74%	62 59%	78 74%	8 78
Klebsiella	80	69 86%	54 68%	59 74%	52 65%	60 75%	66 83%	6 83
Acinetobacter	59	43 73%	40 68%	52 88%	33 56%	43 73%	30 51%	4 78
Escherichia coli	7	3 43%	2 29%	3 43%	2 29%	3 43%	2 29%	2 29

Table: 20 Antibiotic sensitivity pattern of Gram positive cocci

Organisms	No. of Organisms	Penicillin	Ampicillin	Cloxacillin	Oxacillin	Ofloxacin	Ceftazidime
S. aureus	26	6 23%	12 46%	13 50%	18 69%	21 81%	20 77%
CONS	16	8 50%	6 37.5%	10 63%	12 75%	11 69%	12 75%

Table 15 Bacterial isolates at various levels

ORGANISMS	I SWAB (100) - ORGANISMS No. 38						II SWAB (94) - ORGANISMS No. 166					
	Single		Mixed		Total		Single		Mixed		Total	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
Gram negative bacilli						44.64						92.16
Pseudomonas aeruginosa	7	18.42	2	5.26	9	23.61	20	12.04	41	24.69	61	36.73
Klebsiella	4	10.52	2	5.26	6	15.78	13	7.83	38	22.89	51	30.72
Acinetobacter	2	5.26			2	5.26	1	0.6	33	19.87	34	20.47
Escherichia coli	-						4	2.4	3	1.8	7	4.2
Gram positive cocci						55.25						7.82
Staphylococcus aureus	5	13.15			5	13.15	4	2.4	9	5.42	13	7.82
CONS	12	31.57	4	10.52	16	42.1						

APPENDIX

CULTURE MEDIA

BRAIN HEART INFUSION BROTH

Sodium citrate	1gm
Sodium chloride	4gm
Sodium phosphate	5gm
Dextrose	10gm
Peptone	10gm

BRAIN HEART INFUSION

Brain infusion broth	250ml
Heart infusion broth	750ml
Sodium polyanethol sulphonate	0.25gm

Obtain Ox brain and heart. Remove all fat from the heart. Cut into small pieces and grind. Add distilled water three times and keep at 4°C overnight.

From the brain, remove meninges fully and then, weigh. Add distilled water and mash by using hand. Keep in the cooler over night.

Next morning boil the brain and heart separately for 30 minutes. Then filtered through cotton layer. Measure each broth separately. Mix both infusions and the remaining ingredients. Dissolve well and adjust pH of the entire amount to 7.4 to 7.6.

Autoclave at 121°C for 15 minutes. Filter through filter paper and distribute in screw-capped bottles in 50 to 100ml amounts. Autoclave once more at 115°C for 10 minutes.

CHOCOLATE AGAR

Sterile Defibrinated blood - 10ml

Nutrient Agar (melted) - 100ml

When the temperature was about 75°C, sterile blood was added with constant agitation. After addition of blood, kept in water bath and heating was continued till the blood changed to chocolate colour. Cooled to about 50°C and poured about 15ml into Petri dishes with sterile precaution.

MACCONKEY AGAR

Peptone	-2gm
Sodium chloride	-2gm
Bile salt	-0.5gm
Lactose	-1gm
Agar	-1.5gm
Distilled water	-100ml

All the ingredients except lactose were dissolved in distilled water by heating. pH adjusted to 7.61 ml of 1% neutral red solution was added to every 100ml of medium with lactose and sterilized by autoclaving at 121° C for 15 minutes.

MEDIA FOR BIOCHEMILCAL IDENTIFICATION OF BACTERIA

Oxidase	Catalase
Indole	Triple Sugar Iron agar
Citrate	Urease
Methyl Red- Voges Proskauer	Nitrate reduction.
Phenyl alanine deaminase test	Carbohydrate Fermentation media

1.OXIDASE REAGENT

Tetra methyl P- phenlene diamine dihydro chloride -1% aqueous solution.

2.CATALASE TEST

3 % Hydrogen peroxide

3. INDOLE REAGENT

Kovac's reagent

Para dimethyl amino benzaldehyde -10gm

Iso amyl alcohol -150ml

Hydrochloric acid -80ml

4.TRIPLE SUGAR IRON AGAR

Sodium chloride 0.5gm

Yeast extract 0.5gm

Peptone 2gm

Agar 1.5gm

Distilled water 100ml

Distilled by keeping in boiling water bath and the following ingredients were added.

Lactose 1.0gm

Sucrose 1.0gm

Dextrose 0.1gm

Sodium thio sulphate 0.03gm

Ferrous sulphate 0.02gm

pH adjusted to 7.6 phenol red 0.0024gm (2.4 ml of 1% solution) was added and distributed into test tubes in 4ml quantities and autoclaved. The tubes were kept in a slanting position so that to get a deep but and a short slant.

5.SIMMON'S CITRATE AGAR

Sodium chloride 5.0g

Magnesium sulphate 0.2g

Ammonium dihydrogen phosphate 1.0g

Potassium dihydrogen phosphate 1.0g

Sodium citrate 5.0g

Agar 20.0g

Bromothymol blue (1/500 aqueous solution) 40ml

Distilled water 100ml.

The ingredients were mixed and pH adjusted to 6.9 sterilized by autoclaving and poured into tubes as slopes.

6.CHRISTENSEN'S UREA AGAR

Urea Solution

Sodium chloride -5.0g

Dextrose -1.0gm

Trypticase -1.0gm

Mono potassium phosphate -2.0gm

Urea -20.0gm

Distilled water -100gm

Phonol red 1% solution -1.2ml (in alcohol)

UREA AGAR BASE

Agar -1.5 gm

Distilled water -90ml

The ingredients were dissolved in distilled water. pH adjusted to 6.8. Phenol red solution was added & sterilized by filtration. This is stock solution.

Agar was dissolved in distilled water and sterilized by autoclaving. Cooled to 45 degree C and 10ml of urea solution was added, dispensed in 3-5ml quantities and allowed to form a small butt and a long slant.

7.GLUCOSE PHOSPHAT E BROTH (MR-VP medium)

Dipotassium phosphate 5.0gm

Glucose 5.0 gm

Distilled water 100ml

The above ingredients were suspended in distilled water and heated slightly to dissolve them sterilized at 115° C for 15 minutes.

8. POTASSIUM NITRATE BROTH

Potassium nitrate (KNO3) 0.2gm

Peptone 5.0gm

Distilled water 100ml

The above ingredients were mixed and transferred into tubes in 5ml amount and autoclaved.

9. PHENYL ALANINE DEAMINASE TEST

Yeast extract	5g
Disodium hydrogen phosphate	1g
Agar	12g
Di-Phenylalanine	2g
Sodium chloride	5g
Distilled water	lr

pH adjusted to 7.4, distributed in tubes and sterilized by autoclaving at 121° C minutes, allowed to solidify as long slopes.

MEDIA FOR TESTING ANTIBIOTIC SENSITIVITY PATTERN

Mueller-Hinton Agar

Beef extract	20.0gm
Acidicase peptone	7.5gm
Starch	1.5gm
Agar	17.0gm
Distilled water	1000ml

The ingredients were dissolved in one liter of distilled water, mixed thoroughly. Heated with frequent agitation and boiled for 1 minute. pH adjusted to 7.4 +/- 0.2. sterilized by autoclaving and poured in plates.

PROFORMA

Name : D.O.A. :

Age : D.O.D :

Sex : IP NO :

Occupation : Ward No :

Education : Weight :

Marital Status : Time of incident :

Time of starting treatment:

Type of burns : ☐ Chemical ☐ Fire ☐ Electrical ☐ Scald

Degree of the burns :

Depth of the burns : ☐ Superficial ☐ Deep

Type of dressing :

Sites Affected

Head

Neck

Anterior trunk

Posterior trunk

Right buttock

Left buttock

Genitalia

Right upper arm

Left upper arm

Right lower arm

Left lower arm

Right hand

Left hand

Right thigh

Left thigh

Right leg

Left leg

Right foot

Left foot

Microbiological investigations

1. Number of swabs I II III
2. Sites of the swabs
3. Date and Time of taking the swabs
4. Blood Culture

Culture Reports	Microbiology	Antibiogram
Axillae Nose Umbilicus Groin		
Wound swabs I II III Blood culture		

Outcome Of The Patients

ABBREVIATONS

NCCLS - National Committee for Clinical Laboratory Standards.

BAP - Blood Agar Plate.

MAC - MacConkeys Agar Plate.

NAP - Nutrient Agar Plate.

MHA - Mueller Hinton Agar.

MIC - Minimum Inhibitory Concentration.

TBSA - Total Burn Surface Area.

SSD - Silver Sulpha Diazine.

CONS - Coagulate Negative Staphylacoccus.

GPC - Gram Positive Cocci.

GNB - Gram Negative Bacilli.

ESBL - Extended Spectrum Beta Lactamases.

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